**Application of a Bioluminescent Yeast-Reporter System for Screening Chemicals for Estrogenic Effects**

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The Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC) created by the Environmental Protection Agency was mandated with developing methods to screen approximately 87,000 chemicals for biological effects on estrogen, androgen, and thyroid hormone systems. As part of this mandate, EDSTAC proposed that EPA develop rapid, high throughput screening systems to assess a compound's effects on hormonal systems. The Center for Environmental Biotechnology at the University of Tennessee has re-engineered the *Saccharomyces cerevisiae* YES colorimetric estrogen reporter system to produce bioluminescence in response to estrogen or environmental estrogens (*S. cerevisiae* BLYES). Bioluminescence is a reagentless system, eliminating the need for expensive chromophores. Light detection is more sensitive than absorbance detection thus shortening the development time of the assay.

In previous work, strain BLYES was exposed to the estrogenic compounds 17β-estradiol, 17α-estradiol, 17α-ethynyl estradiol, estrone, and 3,4′,5-trichloro-4-biphenylol and compared to the YES assay. The $EC_{50}$ values correlated linearly ($R^2$=0.97) between the two assays. Sensitivities of both assays decreased in the order 17β-estradiol > 17α-ethynyl - estradiol > estrone > 17α-estradiol, with no significant response generated from 3,4′,5-trichloro-4-biphenylol, where the hydroxyl group is stericly hindered by the paired ortho chlorines. The BLYES screen consistently detected estrogenic potencies at 5 to 10-fold lower levels than those attained in the YES assay. Moreover, bioluminescence was detectable in less than 4 hours as compared to 3 days for the colorimetric YES strain.

The primary objective of this research is to validate the BLYES system and develop a standard operating procedure for routine chemical analysis. Work is in progress to test the *S. cerevisiae* BLYES using the proposed 78 substances (ICCVAM, 2002) listed for validation of estrogen receptors and correlate to the colorimetric *S. cerevisiae* YES assay. Parallel research in progress include developing the *S. cerevisiae* BLYES into a standard assay suitable for HTS of chemicals and to modify the *lux* genes for optimum transcription/translation in *S. cerevisiae* thus increasing sensitivity of the assay.